PATENT

ATTORNEY DOCKET NO. TUIN.002.05US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Wigler *et al.*	)	Examiner: Not assigned
Serial No.: Not Assigned	)	Art Unit: Not assigned
Filed:	)	
For: **A REPRESENTATIONAL APPROACH TO DNA ANALYSIS**	)	**CLEAN COPY OF CLAIMS**

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The following are substitute pages 36 – 41, which replace pages 36 – 41 of the original above-referenced patent application, including amendments shown on the attached "Version with Markings to Show Changes Made".

CERTIFICATE OF EXPRESS MAILING

"Express Mail" Label No: EL737340085US

Date of Deposit: Aug 22, 2001

I hereby certify under 37 C.F.R. 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" with sufficient postage on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231

(Signature)

Jeffrey M. Libby

(Printed Name)

CONCLUSION

Should the Examiner have any questions regard the above, in order to expedite prosecution, the Examiner is invited to call the undersigned.

Respectfully submitted,

Dated: August 22, 2001

Barbara Rae-Venter, Ph.D.
Reg. No. 32,750

Rae-Venter Law Group, P.C.
P. O. Box 60039
Palo Alto, CA 94306
Telephone: (415) 328-4400
Facsimile: (415) 328-4477

BRV/JML/jml

WHAT IS CLAIMED IS:

1. A method for producing probes capable of distinguishing at least one sequence difference between DNA from two related different eukaryotic sources, said method comprising:

substantially completely digesting separately the DNA from said two different sources with a restriction endonuclease to provide digested fragments, wherein one of said sources is driver DNA, and the other source is tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises sequence differences between the DNA of said two sources;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested sequences of less than about 2kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

filling in the 3' ends of annealed DNA;

amplifying said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, to provide DNA sequences which serve to identify differences in DNA sequences between said tester source and said driver source.

2. A method according to Claim 1, including the additional step after said filling in of digesting single stranded DNA with a nuclease.

3. A method according to Claim 1, wherein said first round of steps is repeated at least once.

4. A method according to Claim 3, wherein different sets of adaptors are used for at least the first three rounds.

5. A method according to Claim 1, wherein said digesting is with a restriction endonuclease which has a recognition sequence of at least 6 nucleotides and provides a staggered cleavage.

6. A method according to Claim 1, wherein the sources of DNA are cells from related human individuals or the same individual.

7. A method according to Claim 1, wherein said DNA from said two related sources is cDNA.

8. A method according to Claim 1, wherein said DNA from at least one of said two related sources is DNA pooled from a plurality of individual related sources.

9. A method for producing probes capable of distinguishing at least one sequence difference between genomes from two related cellular sources, said method comprising:

substantially completely digesting separately the DNA from said two different sources with a restriction endonuclease having a nucleotide recognition sequence of at least 4 nucleotides, wherein one of said sources is driver DNA, and the other source is tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises sequence differences between the genomes of said two sources;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested sequences of less than about 2kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' end of amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

filling in 3' overhangs;

amplifying said dsDNA with primers to one of said strands of said second set of adaptors to enrich for target DNA;

repeating said first round of steps for at least 2 rounds, using a different set of adaptors in each successive round for said 2 rounds to provide a DNA composition comprising a predominant amount of target DNA;

cloning said DNA composition to provide clones having a substantially homogeneous probe of putative target DNA;

with the proviso that when a plurality of probes of putative target DNA are obtained, optionally including the additional step of:

hybridizing said probes of putative target DNA with driver and tester amplicons, whereby probes of putative target DNA binding to both driver and tester amplicons are discarded.

10. A method according to Claim 9, wherein said related human cellular sources are from the same individual and differ as to the suspected presence of a pathogen.

11. A method according to Claim 9, wherein said related human cellular sources are from the same individual and differ as to the suspected presence of a genetic lesion.

12. A method according to Claim 9, wherein said related human cellular sources are from different individuals.

13. A method for producing probes capable of distinguishing at least one sequence difference between genomes from a neoplastic cell source and a related normal cell source, said method comprising:

substantially completely digesting separately the DNA from said two sources with a restriction endonuclease having a nucleotide recognition sequence of at least 4 nucleotides, wherein said normal cell source is driver DNA, and said neoplastic cell source is tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises sequence differences between the genomes of said two sources comprising at least one of an insertion, deletion, rearrangement or DNA amplification defining target DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set of adaptors to provide amplified amounts of fragments of said digested sequences of less than about 2kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to 5' ends of amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

filling in the 3' ends of overhangs;

amplifying said dsDNA with primers to one of said strands of said second set of adaptors to enrich for target DNA;

repeating said first round of steps for at least 1 additional round, using a different set of adaptors as to the previous round in each successive round to provide a DNA composition comprising a predominant amount of target DNA; and

cloning said DNA composition to provide clones having a substantially homogeneous probe of target DNA.

14. A method for producing probes capable of distinguishing at least one sequence difference between genomes from a neoplastic cell source and a related normal cell source, said method comprising:

substantially completely digesting separately the DNA from said two sources with a restriction endonuclease having a nucleotide recognition sequence of at least 4 nucleotides, wherein said neolastic cell source is driver DNA, and said normal cell source is tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises sequence differences between the genomes of said two sources comprising loss of heterozygosity, homozygosity or hemizygous loss to define target DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set of adaptors to provide amplified amounts of fragments of said digested sequences of less than about 2kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to 5' ends of amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

filling in the 3' ends of overhangs;

amplifying said dsDNA with primers to one of said strands of said second set of adaptors to enrich for target DNA;

repeating said first round of steps for at least 1 round, using a different set of adaptors as to the previous round in each successive round to provide a DNA composition comprising a predominant amount of target DNA; and

cloning said DNA composition to provide clones having a substantially homogeneous probe of target DNA.

15. A kit comprising at least two probes prepared according to the method according to Claim 1.

16. A kit comprising at least two probes prepared according to the method according to Claim 9.

17. A kit comprising at least two probes prepared according to the method according to Claim 13.

18. A kit comprising at least two probes prepared according to the method according to Claim 14.

WHAT IS CLAIMED IS:

19. A DNA probe from a first eukaryotic source of DNA comprising at least one sequence difference from a related second eukaryotic source of DNA, said probe produced according to a method comprising the steps of:

substantially completely digesting separately the DNA from said first and said second source of DNA with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA, and said first source of DNA comprises tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or

successive round, whereby said DNA probe from said first eukaryotic source of DNA is produced.

20. The DNA probe according to claim 19, wherein said first source and said second source of DNA are from the same individual.

21. The DNA probe according to claim 19, wherein one of said first source and said second source of DNA comprises pathogen DNA.

22. The DNA probe according to claim 21, wherein said pathogen DNA is viral DNA.

23. The DNA probe according to claim 19, wherein said DNA is genomic DNA.

24. The DNA probe according to claim 19, wherein said DNA is cDNA.

25. The DNA probe according to claim 19, wherein said DNA probe is cloned.

26. A kit comprising: a DNA probe according to claim 19.

27. A method of identifying sites that differ between two different related eukaryotic DNA sources, said method comprising the steps of:

i) hybridizing a probe obtained according to claim 19 to DNA from a first source and DNA from a second source

ii) determining the sequences to which said probe hybridizes, when said

probe hybridized to the DNA from one but not both DNA sources.

28. A composition comprising a pooled plurality of DNA probes, each probe obtained from a first eukaryotic source of DNA and comprising at least one sequence difference from a related second eukaryotic source of DNA, said probe produced according to a method comprising the steps of:

completely digesting separately the DNA from said first and said second source of DNA with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA, and said first source of DNA comprises tester DNA, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second

round or successive round.

29. A method for detecting at least one genomic alteration occurring in a cancer cell as compared to a normal cell, said method comprising the steps of:

substantially completely digesting separately DNA from a first source and a second source each with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA obtained from a normal cell, and said first source of DNA comprises tester DNA obtained from a cancer cell, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said driver DNA;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, whereby said genomic alteration occurring in a cancer

cell is detected.

30. The method according to Claim 31, wherein said cancer cell and said normal cell are obtained from the same individual.

31. A method of identifying a pathogen DNA sequence incorporated into the genome of a eukaryotic cell, said method comprising the steps of:

substantially completely digesting separately the DNA from a first source and a second source of DNA, each with a restriction endonuclease to provide digested fragments, wherein said second source of DNA comprises driver DNA obtained from an uninfected eukaryotic cell, and said first source of DNA comprises tester DNA obtained from an infected eukaryotic cell, wherein said tester DNA comprises target DNA, wherein said target DNA comprises at least one sequence difference from said;

ligating a first set of adaptors to said digested fragments and amplifying said fragments using primers to one of the strands of said first set adaptors to provide amplified amounts of fragments of said digested fragments of less than about 2 kbp as amplicons;

carrying out a first round of the following steps for enrichment of target DNA:

removing said first set of adaptors from said amplicons and ligating a second set of adaptors to the 5' ends of the amplicons of tester DNA;

combining under melting and annealing conditions said tester amplicons with a large excess of at least about 5-fold of driver amplicons, whereby a portion of the resulting dsDNA comprises self-annealed tester DNA including target DNA;

amplifying said portion of said dsDNA with primers complementary to one of said strands of said second set of adaptors to

enrich for target DNA;

optionally repeating said first round of steps as a second round or successive round, whereby said probe specific for a pathogen DNA sequence is isolated.